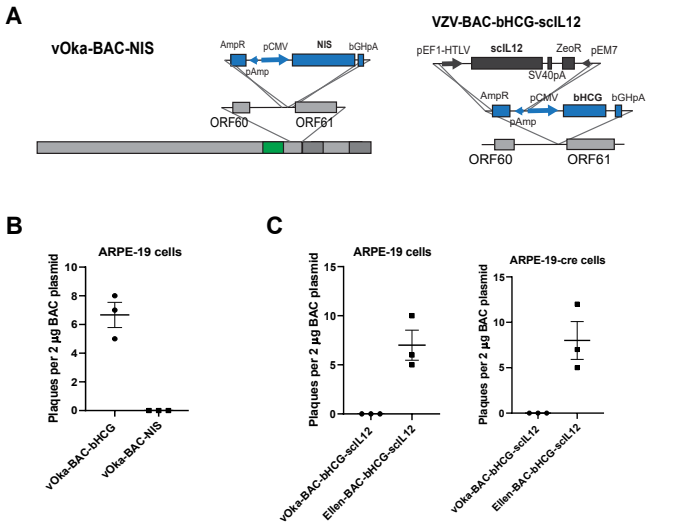
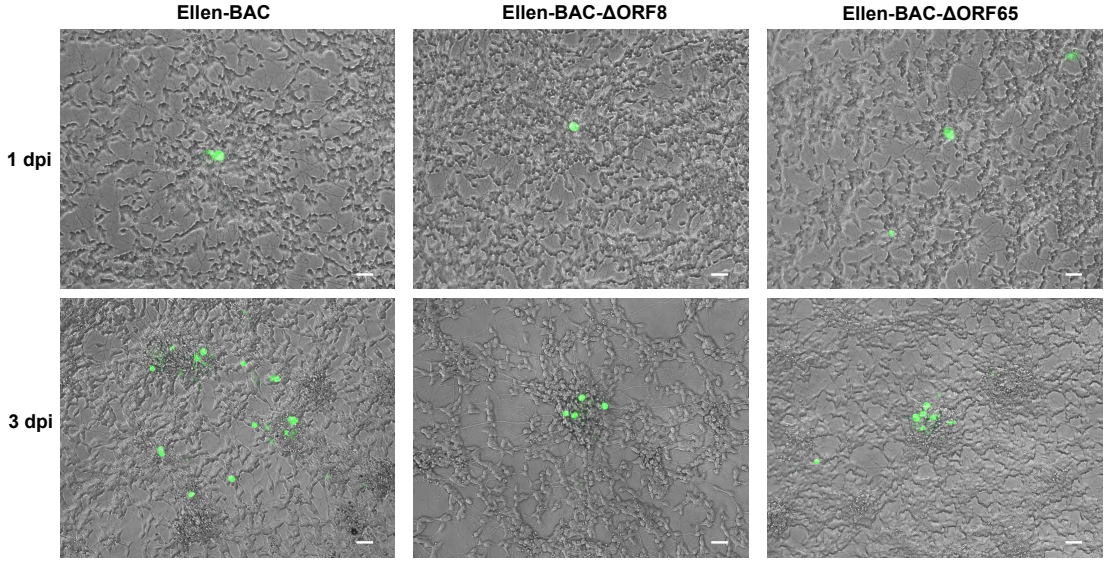
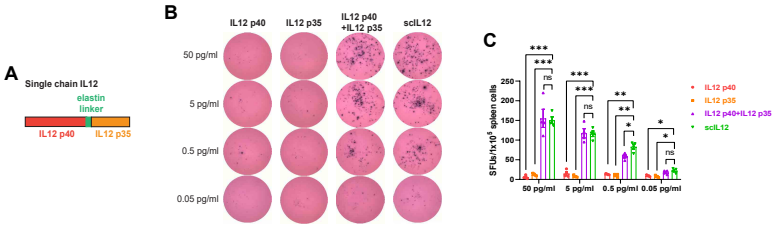


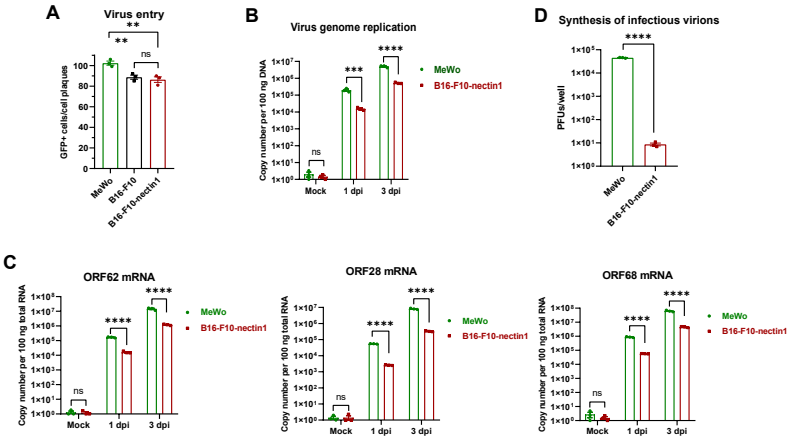
Supplemental Figure 1. Insertion of NIS or scIL12 into vOka genome prevents virus reconstitution. (A) Construction of VZV vOka-BAC-NIS and VZV (vOka or Ellen strain based) -BAC-bHCG-scIL12. **(B)** Reconstitution of vOka-BAC-bHCG and vOka-BAC-NIS. BAC plasmids were transfected into ARPE-19 cells (2 µg per 6×10^5 cells). Green plaques formed on day 5 post-transfection were counted (n=3). **(C)** Reconstitution of vOka-BAC-bHCG-scIL12 and Ellen-BAC-bHCG-scIL12. BAC plasmids were transfected into ARPE-19 or ARPE-19-cre cells (2 µg per 6×10^5 cells). Virus plaques formed on day 5 post-transfection were counted (n=3). scIL12, single chain IL12.



Supplemental Figure 2. Deletion of ORF8 attenuates VZV replication in dSH-SY5Y neuronal cells. The dSH-SY5Y neuronal cells cultured in six-well plates were infected with Ellen-BAC, Ellen-BAC-ΔORF8, or Ellen-BAC-ΔORF65 on day 0 (1000 PFUs per well), and virus infected cells were captured on day 1 and 3. Scale bars = 100 μm.



Supplemental Figure 3. Potency of single chain IL12. (A) Structure of murine single chain IL12 (scIL12). **(B-C)** Potency of IL12 in activating immune cells. The pSelect vector expressing IL12 p40, IL12 p35, or scIL12 was transfected into 293T cells to produce IL12 (group 1: 1µg pSelect-IL12 p40 plasmid into 1×10^6 293T cells; group 2: 1µg pSelect-IL12 p35 plasmid into 1×10^6 293T cells; group 3: 1µg pSelect-IL12 p40 plasmid mixed with 1µg pSelect-IL12 p35 plasmid into 1×10^6 293T cells; group 4: 1µg pSelect-scIL12 plasmid into 1×10^6 293T cells). The supernatant samples were collected on day 4 post transfection, and IL12 concentrations (IL12 p40 in group 1, IL12 p35 in group 2, IL12 p70 in group 3 and group 4) were determined by ELISA analysis. The Mouse IFN-γ Elispot analysis involved exposing 1×10^5 unstimulated mouse spleen cells per well to various concentrations of IL12 (50 pg/ml, 5 pg/ml, 0.5 pg/ml, or 0.05 pg/ml). After a 3-day co-culture, the number of IFN-γ spots formed in each group was counted and compared (n=4, two-way ANOVA with Dunnett's multiple comparisons test). not significant, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.



Supplemental Figure 4. Replication of VZV in B16-F10-nectin1 cells. (A) VZV entry into B16-F10 cells. The MeWo cells, B16-F10 cells, and B16-F10-nectin1 cells cultured in six-well plates (1×10^6 cells per well) were infected with Ellen-BAC-ΔORF8-tet-off-scIL12 virus on day 0 (100 PFUs per well), and agarose overlay was applied to the cell monolayers at 2-hour post infection. On 3 dpi, GFP+ cell plaques (from infected MeWo cells) or separated GFP+ cells/cell plaques (from infected B16-F10 and B16-F10-nectin1 cells) on 3 dpi was counted and compared (n=3, unpaired t-test). **(B-C)** Virus genome replication and viral gene expression. The MeWo cells and B16-F10-nectin1 cells cultured in six-well plates (1×10^6 cells per well) were infected with Ellen-ΔORF8-tet-off-scIL12 virus on day 0 (1000 PFUs per well). On 1 dpi and 3 dpi, infected cells were collected, and total RNA/DNA was extracted. Mock samples without virus infection were collected on 3 dpi as the negative controls. Virus genome copy numbers and mRNA levels of ORF62, ORF28, and ORF68 were determined by qPCR and compared (n=3, multiple t tests). **(D)** Synthesis of infectious virions. The MeWo cells and B16-F10-nectin1 cells cultured in six-well plates were infected with Ellen-ΔORF8-tet-off-scIL12 virus on day 0 (500 PFUs per well). Infected cells were collected on 3 dpi, and titers of infectious viral particles were determined by plaque formation assay in ARPE-19 cells. not significant, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

Supplemental Table 1. List of primers

Primer #	Primer name	Sequence (5'-3')
1	pSG6-F1	ca tgcaagctTATAACTTCGTATAATGTATGCTATACGAAGTTATTTTACAACCCGTGGTGAATTTTTAT
2	pSG6-R1-1	TGTAAGGATCCCTTCACCGGGTAAACAATAATCAAGG
3	pSG6-F2	GATGAGGATCCTTACATAAATACTAAGCCTCTG
4	pSG6-R2	tactcaAGCTTATAACTTCGTATAGCATACATTATACGAAGTTATGTAAATAAACCTTTTATTTTAAGGA
5	pac1-pPGK-NEO-F	tgctcttaattaaTAAGCTTGATATCGAATTCC
6	aat2-pPGK-NEO-R	ttggcgacgtcATATTATGTACCTGACTGAT
7	pac1-PSG6-F	tgctcttaattaatttagcttccttagctcctgaa
8	aat2-PSG6-R	ttggcgacgtctttttttaaggcagttattggt
9	Oka-BAC-R2-F	GTTCCAGATATACCAACCCTTACGACCAATAGCAACACTCAGGTATTTTTAAAATGCACG ttaccaatgcttaatcagtgaggca
10	Oka-BAC-R2-R	ATGCCTTAAACATCCACGGTCTGCTTTATTACCAACTGTATGTAAATTATGATCATTAAA ccatagagcccaccgcatccccagc
11	Oka-BAC-R2.3-F	GTTCCAGATATACCAACCCTTACGACCAATAGCAACACTCAGGTATTTTTAAAATGCACG GGATCTGCGATCGCTCCGGTGCCCG
12	Oka-BAC-R2.3-R	caacgcgtatatctggcccgtacatcgcgaagcagcgcaaaacgcctaaccctaagcaga TTGACAATTAATCATCGGCATAGTA
13	Oka-BAC-R2.6-R	ATGCCTTAAACATCCACGGTCTGCTTTATTACCAACTGTATGTAAATTATGATCATTAAA TTGACAATTAATCATCGGCATAGTA
14	Ellen-BAC-R7.1-F	GTGTATGTTTTTATTTTGTATATAAAATTAAAACATTGCTGGCTGGCGTGGTTATTACAT ATCTGAGGCCCTTTCGTCTTCACTC
15	Galk-replace-VZVgB-2453A-F	TAGCGGGTTTGATCCCGATAAATTTCGAGAAGCCCAGGAAATGATTAAATcctgttgaca attaatcatc ggca
16	Galk-replace-VZVgB-2453A-R	TTTTGCGGGCTTTAGATTCTTGGCGCTCAGCCGCAGATACTAACGTCATA TCAGCACTGTCCTGCTCCTT
17	T-replace-Galk in VZVgB-2453A	TAGCGGGTTTGATCCCGATAAATTTCGAGAAGCCCAGGAAATGATTAAATTTATGACGTTAGTATCTGCGGCTGAGCGCCAAGAATCTAAAGCCCGCAAAAAAAATAAG
18	Ellen-BAC-R6.1-F	GTGTATGTTTTTATTTTGTATATAAAATTAAAACATTGCTGGCTGGCGTGGTTATTACAT CCTGTTGACAATTAATCATCGGCAT
19	Ellen-BAC-R6.1-R	TTCACAAAAAAATAAAATTTGCATCGTTATTAATATGAACGAAGCGGTAATTGATCCCAT TCAGCACTGTCCTGCTCCTTGTGAT
20	Ellen-BAC-R6.1	GTGTATGTTTTTATTTTGTATATAAAATTAAAACATTGCTGGCTGGCGTGGTTATTACATATGGGATCAATTACCGCTTCGTTCATATTAATAACGATGCAAATTTTATTTTTTTGTGAA
21	Ellen-BAC-R6.3-F	AATCGCGTTACCAAACTCTTCTTCCGTATCCGTAGATTCCGAGTCCTCGAAATCGTCCAC CCTGTTGACAATTAATCATCGGCAT
22	Ellen-BAC-R6.3-R	CAAAAGGTGTGGTATTAAACACTTTTAAACTAGCCTGCTGGCCTTACAATCTGGATTTCT TCAGCACTGTCCTGCTCCTTGTGAT
23	Ellen-BAC-R6.3	AATCGCGTTACCAAACTCTTCTTCCGTATCCGTAGATTCCGAGTCCTCGAAATCGTCCACAGAAATCCAGATTGTAAGGCCAGCAGGCTAGTTTAAAAGTGTTTAATACCACACCTTTTG
24	Ellen-BAC-R7-R	TTCACAAAAAAATAAAATTTGCATCGTTATTAATATGAACGAAGCGGTAATTGATCCCAT TTGACAATTAATCATCGGCATAGTA
25	Ellen-BAC-R7.2-F	GGGGGAGGTGTGGGAGGTTTTTTAAAGCAAGTAAAACCTCTACAAATGTGGTATGgaatt CCTGTTGACAATTAATCATCGGCAT
26	Ellen-BAC-R7.2-R	TTCACAAAAAAATAAAATTTGCATCGTTATTAATATGAACGAAGCGGTAATTGATCCCAT CATGTCTGGATCCTTACTTAGTTAC
27	VZV-qORF28-F	GTA TCC ACC TCG GCT TTG TT
28	VZV-qORF28-R	GAC CGC TTG GTA CCG TTA TT
29	ORF62-F	ATT ACA GGC GAG CCC ATT AG
30	ORF62-R	GAC TTC AAC CAG AAC CCA GAA
31	ORF28-F	GTA TCC ACC TCG GCT TTG TT
32	ORF28-R	GAC CGC TTG GTA CCG TTA TT
33	ORF68-F	GCG GAG TCT TCA TGG GTA AAT
34	ORF68-R	GTG TTC GTG TGC GTT CTC TA